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Three Retroviral Sequences in Amphibians Are Distinct from Those in Mammals and Birds

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We isolated and characterized three endogenous retroviral fragments from the dart-poison frog *Dendrobates ventrimaculatus*. These are the first retroviral sequences to be identified in amphibians, and consequently retroviruses have now been found in each of the five major vertebrate classes. Comparison of the amphibian retroviral fragments, termed DevI, DevII, and DevIII, with mammalian and avian isolates revealed significant differences between their nucleotide sequences. This suggested that they were only distantly related to the seven currently recognized retroviral genera. Additional analysis by phylogeny reconstruction showed that the amphibian retroviral fragments were approximately equally related to the Moloney leukemia-related viruses, the spumaviruses, and walleye dermal sarcoma virus. Hybridization experiments revealed that viruses closely related to DevI, DevII, and DevIII do not appear to be widespread in other vertebrates and that DevI, DevII, and DevIII are all present at high copy numbers within their amphibian hosts, typically at over 250 copies per genome. The viruses described here, along with two others which have recently been found in a fish and a reptile, indicate that there may be some major differences in the retroviruses harbored by different vertebrate classes. This suggests that further characterization of retroviruses of fish, reptiles, and amphibians will help in understanding the evolution of the whole retroviral family and may well lead to the discovery of retroviruses with novel biological properties.

The retroviruses are a family of RNA viruses which are associated with a variety of vertebrate diseases (10). Numerous examples have been isolated from mammalian and avian hosts, and many have been extensively studied. On the basis of their biological and phylogenetic properties, retroviruses from these two vertebrate classes have been classified into seven related groups or genera (5–7, 10, 38). All of the genera are known to have exogenous (horizontally transmitted) members, and several also have endogenous counterparts (vertically transmitted elements which are integrated into the host genome) with a wide distribution.

In contrast to the data which are available for retroviruses of mammals and birds, there is little information regarding the diversity and distribution of retroviruses in lower vertebrates. There have been reports of viral particles in several species of fish and reptiles, but the almost complete absence of sequence information has hindered attempts at understanding their relationships with the known genera (1, 8, 11, 17, 18, 26, 39). The molecular data that are available from the tuatara (*Sphenodon* sp.), a reptile, and the walleye (*Stizostedion vitreum*), a fish, suggest that at least some lower-vertebrate retroviruses are substantially different from those found in mammals and birds (13, 21, 22, 37). There have been no reports to date of retroviruses or retrovirus-like particles in amphibians.

Retroviruses have highly variable nucleotide sequences, and thus the investigation of evolutionary relationships between distantly related retroviruses has been based almost exclusively on the regions of their genomes which have the highest levels of sequence conservation (6, 38). These regions, which are present in the reverse transcriptase gene (and to a lesser extent in the protease and RNase H genes), have significant sequence homology across all the retroviruses studied to date and also between retroviruses and other related retroelements. Several recent studies have used a 178-codon region of the reverse transcriptase gene which extends across seven domains (6, 38) to determine relationships among retroviruses.

We used PCR to investigate whether retroviruses were present within the genome of the amphibian *Dendrobates ventrimaculatus* (Amazonian dart-poison frog), a member of the family Dendrobatidae. The members of the Dendrobatidae, or dart-poison frogs (so called because toxic alkaloids secreted from glands in their skin are used to make poison darts), are small terrestrial frogs which inhabit forested areas in the neotropics, specifically from Nicaragua to central Brazil. An aliquot of genomic DNA, pooled from two individuals, was obtained from *D. ventrimaculatus*. This DNA was then used in a PCR which used two degenerate oligonucleotide primers [5′ GT(T/G)TTI(G/T)TIGA(T/C)ACIGGI(G/T)C and 5′ ATIA GIA(G/T)(A/G)TC(A/G)TCIAC(A/G)T, where I indicates inosine], designed to be specific for conserved motifs in retroviral protease and reverse transcriptase genes. Primers based on these motifs amplify relatively large retroviral fragments (approximately 800 bp to 1 kb) from a wide range of vertebrate taxa (unpublished data). They therefore allow information encoded by the protease gene and five of the seven highly conserved domains within the reverse transcriptase gene to be included in subsequent sequence analysis. The PCR mixture contained 0.5 μg of template DNA, 150 pmol of each primer, and 2 U of Cetus *Taq* polymerase in a buffer consisting of 200 μM (each) deoxynucleoside triphosphates, 50 mM KCl, 10 mM Tris (pH 8.0), and 1.5 mM $MgCl_2$. Samples were subjected to 35 cycles with the following parameters: annealing at 45°C for 30 s, extension at 72°C for 1 min, and denaturation at 94°C for 20 s.

Three products, of approximately 700 to 1,000 bp, were observed in the *Dendrobates* sample after gel electrophoresis.

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The products were excised, cloned into the plasmid vector pCRII (Invitrogen), and sequenced with a Sequenase kit (United States Biochemical). Sequence analysis demonstrated that all the products, designated *D. ventrimaculatus* endogenous virus types I, II, and III (DevI, DevII, and DevIII), contained regions with homology to previously described retroviral reverse transcriptase genes. However, additional analysis showed that both DevII and DevIII contained substantial deletions. Sequence information for these regions was obtained by designing two pairs of internal oligonucleotide primers (DevII, 5′ TCAGGCCACCATACAGTACT and 5′ ATC GTCGACGTACTGTAATAG; DevIII, 5′ TCAAGTCAGA CATTCCCAGC and 5′ ATGTGACCCAGTTGGTGAAC) specific for suitable regions of the DevII and DevIII fragments. These primers were then used in PCRs with aliquots of *Dendrobates* genomic DNA as the template. Reaction conditions, cycling parameters, cloning, and sequencing were as described above.

The retroviral fragments were aligned with each other as shown in Fig. 1. The sequences showed features typical of many other previously described retroviruses, such as the presence of the reverse transcriptase gene immediately downstream of the protease gene. Furthermore, the fragments were also similar in size (926 to 972 bp, including the primer sequences) to the homologous regions in other retroviruses, which vary between approximately 800 bp in the lentiviruses and 960 bp in the Moloney leukemia virus (MLV)-related viruses.

We next constructed an amino acid alignment which included examples of all the previously described retroviral genera (Fig. 2). The alignment, 177 amino acids in length, was based on that described by Xiong and Eickbush (38) and extended from 24 residues upstream of reverse transcriptase domain 1 to the center of domain 5. Scores for similarity between the *Dendrobates* fragments and the other retroviruses were then calculated from the alignment, as shown in Table 1. DevI, DevII, and DevIII did not appear to be closely related to any of the retroviruses surveyed. The greatest similarity (40 to 50%) was obtained with walleye dermal sarcoma virus (WDSV), and a relatively high score (38 to 47%) was also observed with gibbon ape leukemia virus, which is a member of the MLV-related viruses. It was also apparent that the amphibian retroviral fragments were relatively distinct from each other, with only 45 to 59% similarity. For comparison, these scores were slightly lower than those obtained when distantly related members of two known genera were compared: equine infectious anemia virus and simian immunodeficiency virus-macaque (two lentiviruses) were 57% similar, and human endogenous retrovirus E and gibbon ape leukemia virus (two MLV-related viruses) were 61% similar.

To investigate the affinities of the amphibian retroviral fragments further, we constructed phylogenetic trees by using the PAUP and PHYLIP packages (9, 36). Maximum parsimony and two distance-based methods (neighbor joining and Fitch-Margoliash) were initially used to perform the analyses. Maximum parsimony trees were constructed from amino acid sequences and were based on the alignment shown in Fig. 2 (the regions between the reverse transcriptase domains were omitted). Multiple runs were performed with different parameters, such as stepwise addition and branch-swapping options, in order to find the minimum tree. Nucleotide sequences, aligned identically to the amino acids shown in Fig. 2, were used to generate trees by using the neighbor-joining and Fitch-Margoliash approaches. In some reconstructions, third codon positions were deleted, whereas transition/transversion ratios were varied in others. Global search and random addition options were also invoked where appropriate. Trees based on data sets reduced by removing one or more retroviral isolates from the analysis were also generated. We found that there were three possible relationships of DevI, DevII, and DevIII to other retroviruses. The first, shown in Fig. 3, placed all three as sister taxa to a spumavirus-Sphenodon endogenous virus (SpeV)-WDSV lineage. This topology was obtained with all the neighbor-joining trees we generated, except those which excluded the WDSV sequence from the analysis. When this isolate was removed from the data set, the vast majority of trees placed the amphibian retroviral fragments as sister taxa to the MLV genus. The third and final topology which we observed resulted from the construction of some maximum parsimony trees, in which DevI, DevII, and DevIII were basal to an MLV-spumavirus lineage. It is interesting that the relationship of WDSV to other retroviruses was equally dependent on the inclusion of SpeV and the inclusion of the amphibian sequences. Without them, it appeared as a basal member of the MLV-related viruses, in agreement with the tree reported by Holzschu et al. (13). We used the maximum likelihood-based program DNAML from the PHYLIP package to try to determine which of these relationships was the most probable (9). Although this method demonstrated that the best tree was the same as that shown in Fig. 3, the others were not significantly less likely.

Clearly there are at present too few sequences, with too much diversity, in this region of the retroviral family tree for the exact relationships of DevI, DevII, and DevIII (and WDSV) to be determined. We therefore think it prudent at present (until additional sequences become available) to regard this region of the tree as comprising four approximately equally related groups; (i) the MLV-related viruses, (ii) the spumaviruses and SpeV, (iii) WDSV, and (iv) DevI to -III.

The distributions and copy numbers of the amphibian retroviral fragments were investigated by hybridization analysis. Aliquots of *Eco*RI-digested DNA (10 μg) from seven amphibians were hybridized to 25 ng of [^{32}P]dCTP-labelled DevI, DevII, or DevIII probe as described by Sambrook et al. (31) (Fig. 4). Hybridization was performed at 65°C with Denhardt's solution and salmon sperm DNA as the blocking agents. The filters were washed at 65°C in the presence of 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.5% sodium dodecyl sulfate (SDS). Two of the samples used in the hybridizations were from *D. ventrimaculatus* and *Minyobates minutus* (the minute dart-poison frog, another member of the Dendrobatidae). As expected, all three probes hybridized to the *Dendrobates* sample. In two cases, DevII and DevIII (Fig. 4b and c, respectively), most of the signal was present in a single band at approximately 5 kb. Weak bands were also visible in the *Minyobates* sample in both cases. In contrast, DevI (Fig. 4a) hybridized with approximately equal intensities to the *Dendrobates* and *Minyobates* samples, without any strong banding patterns. This suggested that either the genomic sequences with homology to DevI were present at high copy numbers or the samples to which the probe was hybridized were degraded. To rule out the latter possibility, the filter to which the DevII probe had been hybridized was stripped and rehybridized to the DevI retroviral fragment under conditions identical to those described above. The same hybridization pattern was again observed (unpublished data). None of the probes hybridized to DNA obtained from the other five amphibians (tiger salamander [*Ambystoma tigrinum*], African clawed toad [*Xenopus laevis*], edible frog [*Rana esculenta*], European common frog [*Rana temporaria*], and leopard frog [*Rana pipiens*]). Hybridization of each of the probes to DNA isolated from a garter snake (*Thamnophis sirtalis*), a rook (*Corvus frugilegus*), and a horse (*Equus caballus*) also failed to show

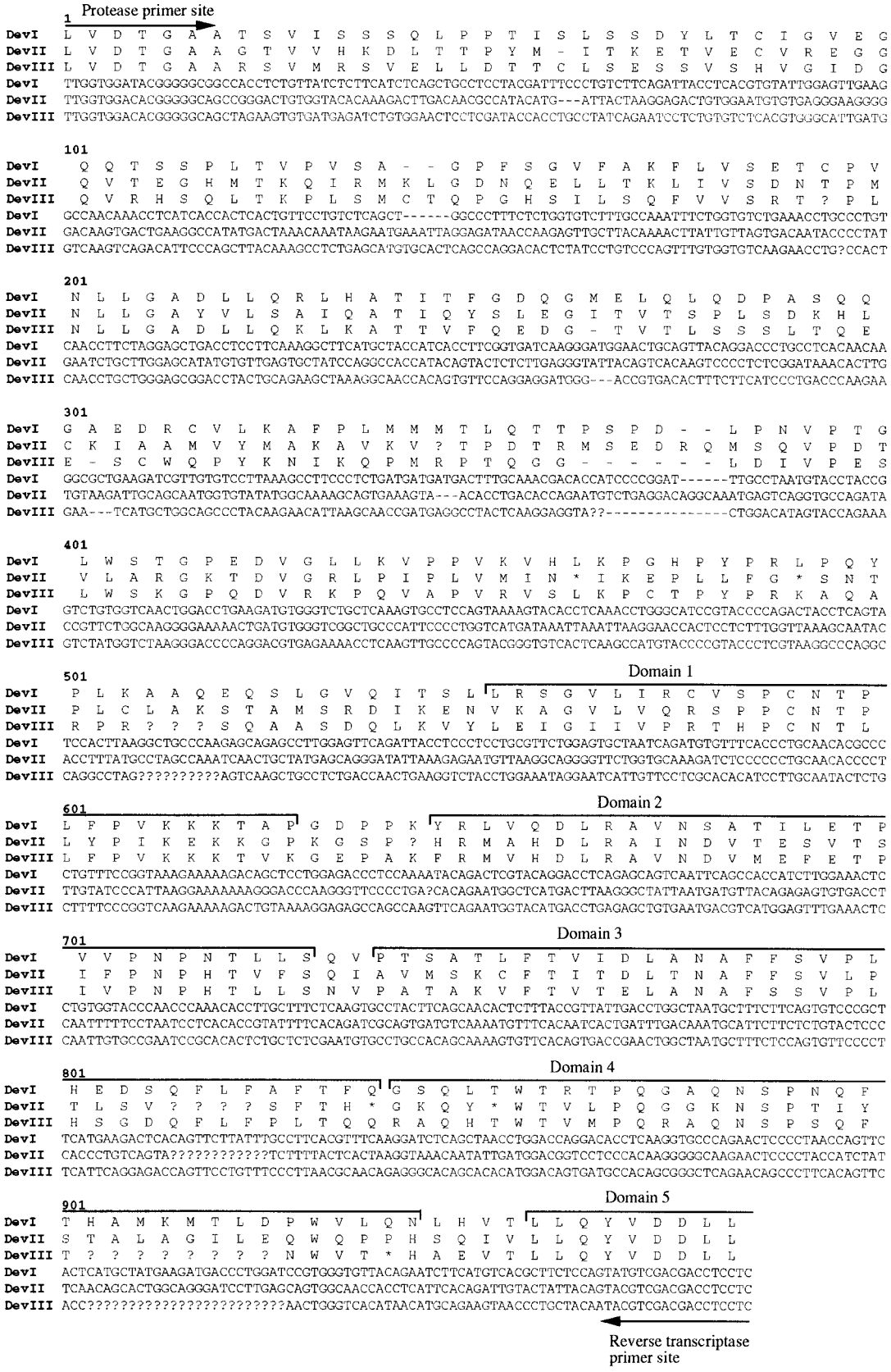

FIG. 1. Nucleotide sequence alignment and amino acid translation of the three *Dendrobates* retroviral fragments, DevI, DevII, and DevIII. Gaps introduced into the sequences to allow optimal alignment are denoted by dashes. Frameshifts and stop codons are represented by question marks and asterisks, respectively. The nucleotide sequences from positions 318 to 582 (DevII) and 789 to 937 (DevIII) were derived from separate clones because of deletions in the first clones examined.

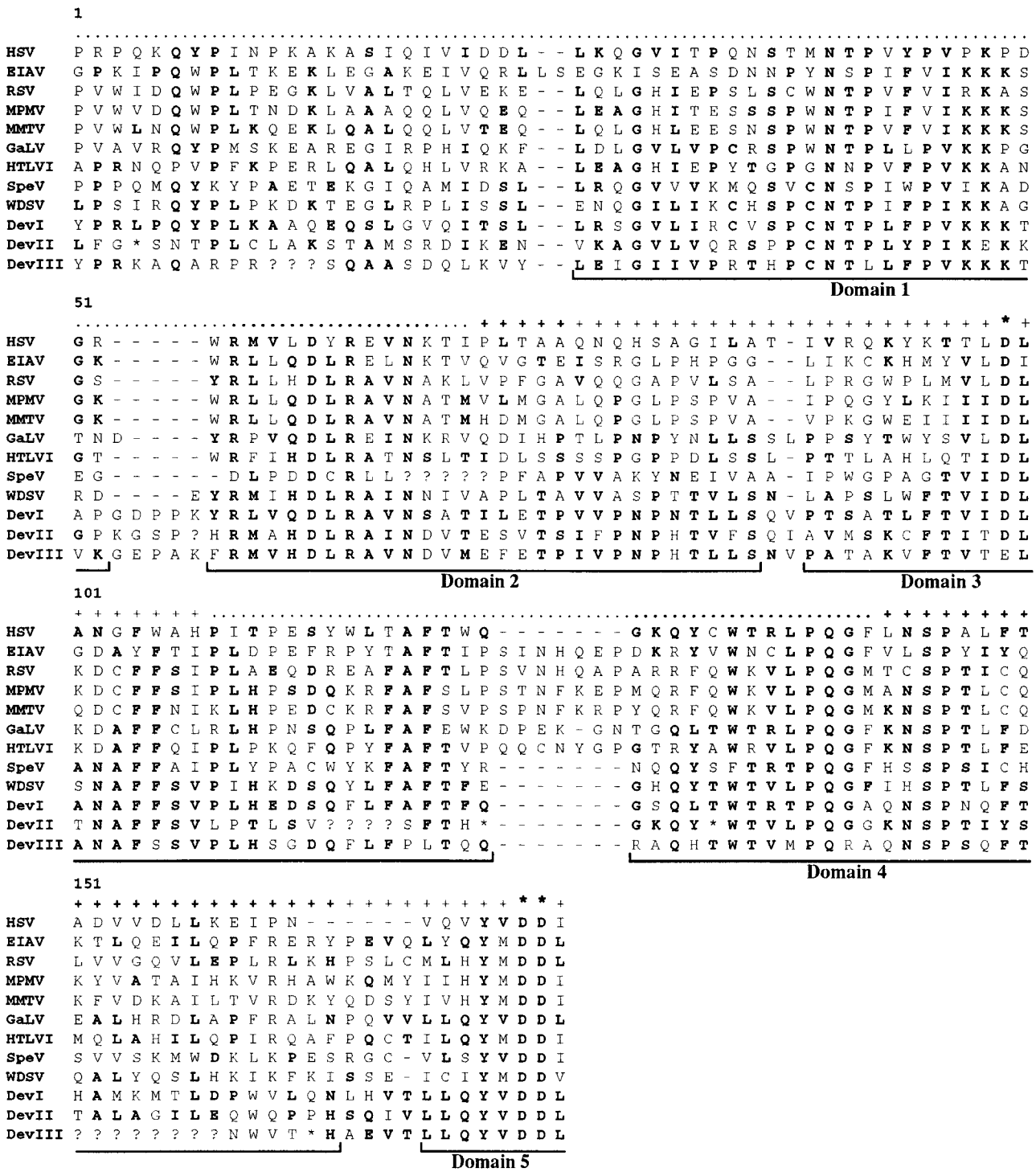

FIG. 2. Amino acid sequence alignment of DevI, DevII, and DevIII with representative members of each of the seven recognized retroviral genera. The recently identified reptilian retrovirus SpeV and the piscine retrovirus WDSV are also included. Amino acids conserved between one or more of the *Dendrobates* viral fragments and other retroviruses are in boldface type. Frameshifts and stop codons are indicated as described in the legend to Fig. 1. Shown in relation to the conserved amino acid domains are the finger and palm regions identified from structural analysis of the human immunodeficiency virus type 1 reverse transcriptase protein (14, 15). The finger regions (identified by dots above the alignment) have been implicated in the binding and positioning of the template strand (especially positions 59 to 69), whereas the palm (represented by plus signs) is necessary for template, primer, and nucleoside triphosphate binding (positions 72 to 76 and 130 to 164) and catalysis. The three residues which are thought to make up the active site are indicated by asterisks (positions 99, 174, and 175). HSV, human spumaretrovirus (23); EIAV, equine infectious anemia virus (35); RSV, Rous sarcoma virus (32); MPMV, Mason-Pfizer monkey virus (34); MMTV, mouse mammary tumor virus (24); GaLV, gibbon ape leukemia virus (3); HTLVI, human T-cell lymphotropic virus type 1 (19).

TABLE 1. Percent amino acid similarities between DevI, DevII, and DevIII and isolates from other retroviral genera

Dendrobates retroviral fragment	% Amino acid similarity to[a]:										
	HSV	EIAV	RSV	MMTV	GaLV	HTLV-1	SpeV	WDSV	DevI	DevII	DevIII
DevI	36	28	31	32	47	39	38	50		45	59
DevII	33	30	30	33	39	35	28	43	45		47
DevIII	31	23	28	28	38	36	28	40	59	47	

[a] HSV, human spumaretrovirus; EIAV, equine infectious anemia virus; RSV, Rous sarcoma virus; MMTV, mouse mammary tumor virus; GaLV, gibbon ape leukemia virus; HTLV-1, human T-cell lymphotropic virus type 1.

any signal (unpublished data). Furthermore, the DevII fragment was hybridized to a panel of DNA samples from mammals (cat [*Felis catus*], human [*Homo sapiens*], sheep [*Ovis aries*], and Mexican free-tailed bat [*Tadarida brasiliensis*]) and birds (wood pigeon [*Columba palumbus*], sparrow [*Passer domesticus*], pheasant [*Phasianus colchicus*], and partridge [*Perdix perdix*]), again without any obvious signal. It therefore appears that retroviruses similar in sequence to DevI, DevII, and DevIII have a relatively restricted range within vertebrate genomes.

Although the copy number of each of the three fragments could not be estimated directly from the Southern hybridizations, the relatively strong signal obtained (which was over 150 cps, as measured with a hand-held minimonitor) suggested that DevI, DevII, and DevIII were all present at high copy numbers.

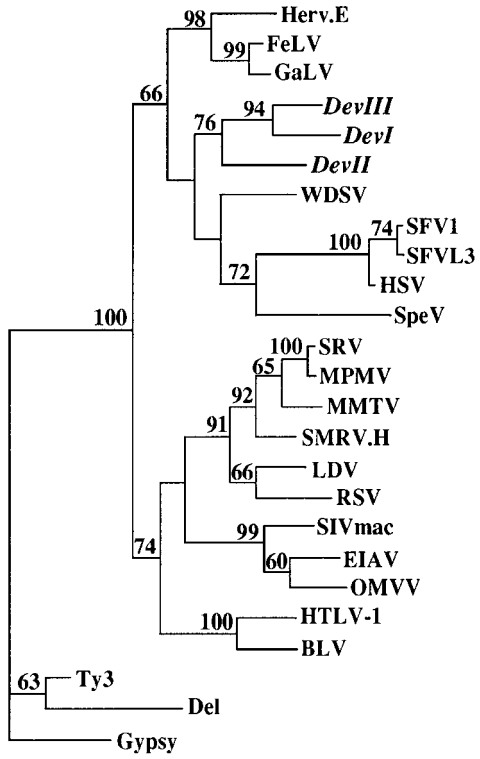

FIG. 3. Neighbor-joining tree of retroviral reverse transcriptase sequences based on the alignment shown in Fig. 2. Three *gypsy* long terminal repeat-retrotransposon sequences (*gypsy*, *Del*, and Ty3) were included as outgroups. The branch lengths are proportional to the distances between the taxa. Each value is the percent support for a particular node after 100 bootstrap replicates were performed. References and abbreviations are as in the legend to Fig. 2, except for feline leukemia virus (FeLV) (4), human endogenous retrovirus E (Herv.E) (29), simian foamy virus type 1 (SFV1) (16), simian foamy virus type 3 (SFVL3) (28), simian immunodeficiency virus-macaque (SIVmac) (2), bovine leukemia virus (BLV) (30), *gypsy* (20), *Del* (33), Ty3 (12), simian retrovirus (SRV) (27a), squirrel monkey retrovirus (SMRV.H) (2a), lymphoproliferative disease virus (LDV) (11a), ovine maedi-visna virus (OMVV) (27b).

To investigate this further, we performed dot blot hybridizations (27). Defined amounts of each of the cloned retroviral fragments and genomic DNA from *D. ventrimaculatus* or *M. minutus* were spotted onto a nylon membrane and then fixed. The membranes were screened with the DevI, DevII, and DevIII fragments (which had been excised from the plasmid vector, gel purified, and labelled with [^{32}P]dCTP) as probes. Hybridization and washing conditions were the same as those described for the Southern hybridizations. Retroviral copy number could then be calculated by using the estimated genome size of the host species and the relative intensity of the hybridization signal obtained from the cloned retroviral fragments and genomic DNA. The exact sizes of the *D. ventrimaculatus* and *M. minutus* genomes are unknown, but they could be estimated from the known sizes of genomes of other frogs, the great majority of which contain between 4.8 and 21.1 pg of DNA per cell (25). These figures were used to calculate the approximate copy numbers of DevI, DevII, and DevIII in the two host species. The copy number of DevI was estimated to be 250 to 1,000 copies per genome in *D. ventrimaculatus* and 500 to 2,000 copies per genome in *M. minutus*. The copy number of DevII in *D. ventrimaculatus* was estimated to be 250 to 1,000 copies per genome, and that of DevIII in *D. ventrimaculatus* was estimated to be 1,000 to 4,000 copies per genome. Both the lower and upper genome size values were used, and thus these figures reflect the lower and upper copy number estimates. It was apparent that the three retroviral fragments were all present at high copy numbers of at least 250 copies per genome, with a maximum estimate of 4,000 copies per genome for DevIII in *D. ventrimaculatus.*

DevI, DevII, and DevIII are all clearly members of the family *Retroviridae* and as such are the first retroviruses to be isolated from amphibian genomes. Our phylogenetic analysis suggested that the relationship of the three *Dendrobates* retroviral fragments cannot yet be determined exactly, although it is clear that they are more closely related to the spumaviruses, MLV-related viruses, and WDSV than to members of the other retroviral genera. The lack of a clear phlogenetic relationship to other retroviruses suggests that DevI, DevII, and DevIII should not at present be assigned to any of the currently recognized retroviral genera. This proposal is supported by the percent amino acid homologies between the *Dendrobates* retroviruses and other retroviruses. The highest homologies (between 38 and 50%, with the MLV-related viruses and WDSV) are lower than those between the most divergent members of any of the known retroviral genera. For example, two distantly related MLV-related viruses, gibbon ape leukemia virus and human endogenous retrovirus E, have 61% amino acid homology across the region shown in Fig. 2.

The highly divergent nature of the *Dendrobates* retroviral fragments, in comparison with the retroviruses which have

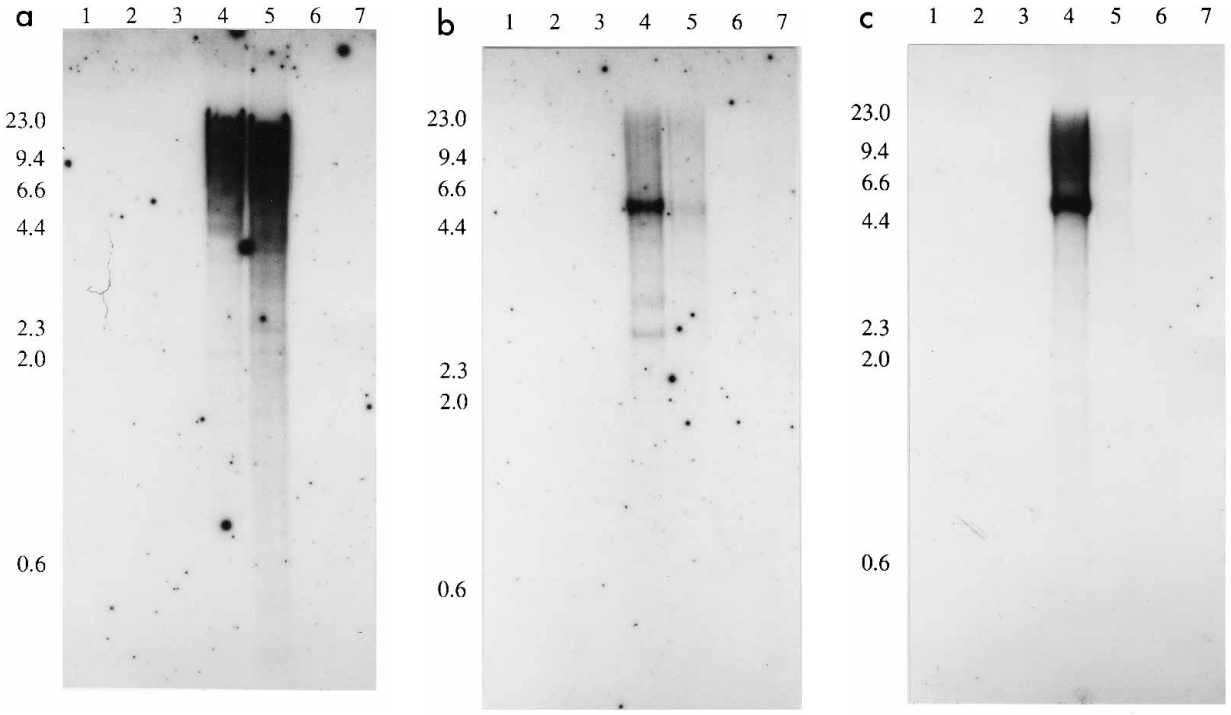

FIG. 4. Southern blot analyses of *Eco*RI-digested genomic DNA hybridized to the retroviral fragments DevI (a), DevII (b), and DevIII (c). Hybridization was at 65°C for 16 h. Filters were washed in 0.5× SSC–0.5% SDS, also at 65°C. Lanes 1, leopard frog (*R. pipiens*); lanes 2, edible frog (*R. esculenta*); lanes 3, European common frog (*R. temporaria*); lanes 4, Amazonian dart-poison frog (*D. ventrimaculatus*); lanes 5, minute dart-poison frog (*M. minutus*); lanes 6, African clawed toad (*X. laevis*); lanes 7, tiger salamander (*A. tigrinum*). Sizes are indicated in kilobases.

been isolated from mammals and birds, is a feature they share with the two other lower-vertebrate retroviruses for which molecular data have been reported. The type C retrovirus WDSV from walleyes and SpeV from tuataras are also sufficiently distinct to hinder their inclusion in any of the recognized retroviral genera (13, 37). It is therefore becoming apparent that the retroviruses harbored by fish, reptiles, and amphibians represent a novel, unexplored pool of retroviral diversity. Further characterization of this diversity (by sequencing full-length proviral clones, for example) may well lead to the discovery of novel retroviral genes involved in pathogenesis or replication and provide a better understanding of the evolution of the whole retroviral family.

Nucleotide sequence accession numbers. The nucleotide sequences presented here will appear in the EMBL, GenBank, and DDBJ sequence databases under accession numbers X95795 (DevI), X95796 (DevII), and X95797 (DevIII).

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REFERENCES

1. **Carneiro, S. M., H. Tanaka, J. J. Kisielius, and A. Sesso.** 1992. Occurence of retroviral-like particles in various cellular and intracellular compartments of the venom glands from *Bothrops jararacussu*. Res. Vet. Sci. **53:**399–401.
2. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature (London) **328:**543–547.

2a. **Chiu, I.-M., R. Callahan, S. R. Tronick, J. Schlom, and S. A. Aaronson.** 1984. Major pol gene progenitors in the evolution of oncoviruses. Science **233:** 364–370.

3. **Delassus, S., P. Sonigo, and S. Wain-Hobson.** 1989. Genetic organization of gibbon ape leukemia virus. Virology **173:**205–213.
4. **Donahue, P. R., E. A. Hoover, G. A. Beltz, N. Riedel, V. M. Hirsch, J. Overbaugh, and J. I. Mullins.** 1988. Strong sequence conservation among horizontally transmissible, minimally pathogenic feline leukemia viruses. J. Virol. **62:**722–731.
5. **Doolittle, R. F., and D.-F. Feng.** 1992. Tracing the origin of retroviruses. Curr. Top. Microbiol. Immunol. **176:**195–211.
6. **Doolittle, R. F., D.-F. Feng, M. S. Johnson, and M. A. McClure.** 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64:**1–30.
7. **Doolittle, R. F., D.-F. Feng, M. A. McClure, and M. S. Johnson.** 1990. Retrovirus phylogeny and evolution. Curr. Top. Microbiol. Immunol. **157:** 1–18.
8. **Eaton, W. D., B. Folkins, J. Bagshaw, G. Traxler, and M. L. Kent.** 1993. Isolation of a retrovirus from two fish lines developed from chinook salmon (*Oncorhynchus tshawytscha*) with plasmacytoid leukaemia. J. Gen. Virol. **74:**2299–2302.
9. **Felsenstein, J.** 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics **5:**164–166.
10. **Francki, R. I. B., C. M. Fauquet, D. L. Knudson, and F. Brown (ed.).** 1991. Classification and nomenclature of viruses. Fifth report of the International Committee on Taxonomy of Viruses. Arch. Virol. **1991**(Suppl. 2)**:**290–298.
11. **Frerichs, G. N., D. Morgan, D. Hart, C. Skerrow, R. J. Roberts, and D. E. Onions.** 1991. Spontaneously productive C-type retrovirus infection in fish cell lines. J. Gen. Virol. **72:**2537–2539.

11a. **Gak, E., A. Yanev, L. Sherman, M. Ianconescu, S. R. Tronik, and A. Gazit.** 1991. Lymphoproliferative disease virus of turkeys: sequence analysis and transcriptional activity of the long terminal repeat. Gene **99:**157–162.

12. **Hansen, L. J., D. L. Chalker, and S. B. Sandmeyer.** 1988. Ty3, a yeast retrotransposon associated with tRNA genes, has homology to animal retroviruses. Mol. Cell. Biol. **8:**5245–5256.
13. **Holzschu, D. L., D. Martineau, S. K. Fodor, V. M. Vogt, P. R. Bowser, and J. W. Casey.** 1995. Nucleotide sequence and protein analysis of a complex piscine retrovirus, walleye dermal sarcoma virus. J. Virol. **69:**5320–5331.
14. **Jacobo-Molina, A., J. Ding, R. G. Nanni, A. D. Clark, X. Lu, C. Tantillo, R. L. Williams, G. Kamer, A. L. Ferris, P. Clark, A. Hizi, S. H. Hughes, and E. Arnold.** 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0Å resolution shows dent DNA. Proc. Natl. Acad. Sci. USA **90:**6320–6324.
15. **Kohlstaedt, L. A., J. Wang, J. M. Friedman, P. A. Rice, and T. A. Steitz.** 1992. Crystal structure at 3.5Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science **256:**1783–1790.
16. **Kupiec, J. J., A. Kay, M. Hayat, R. Ravier, J. Peries, and F. Galibert.** 1991.

Sequence analysis of the simian foamy virus type 1 genome. Gene **101:**185–194.
17. **Lunger, P. D., and H. F. Clark.** 1977. Intracytoplasmic type A particles in viper spleen cells. J. Natl. Cancer Inst. **58:**809–811.
18. **Lunger, P. D., W. D. Hardy, and H. F. Clark.** 1974. C-type particles in a reptilian tumor. J. Natl. Cancer Inst. **52:**1231–1235.
19. **Malik, K. T., J. Even, and A. Karpas.** 1988. Molecular cloning and complete nucleotide sequence of an adult T cell leukaemia virus/human T cell leukaemia virus type 1 (ATLV/HTLVI) isolate of Caribbean origin: relationship to other members of the ATLV/HTLVI subgroup. J. Gen. Virol. **69:** 1695–1710.
20. **Marlor, R. L., S. M. Parkhurst, and V. G. Corces.** 1986. The *Drosophila melanogaster* gypsy transposable element encodes putative gene products homologous to retroviral proteins. Mol. Cell. Biol. **6:**1129–1134.
21. **Martineau, D., P. R. Bowser, R. R. Renshaw, and J. W. Casey.** 1992. Molecular characterization of a unique retrovirus associated with a fish tumor. J. Virol. **66:**596–599.
22. **Martineau, D., R. Renshaw, J. R. Williams, J. W. Casey, and P. R. Bowser.** 1991. A large unintegrated retrovirus DNA species present in a dermal tumor of walleye *Stizostedion vitreum*. Dis. Aquat. Org. **10:**153–158.
23. **Maurer, B., H. Bannert, G. Darai, and R. M. Flügel.** 1988. Analysis of the primary structure of the long terminal repeat and the *gag* and *pol* genes of the human spumaretrovirus. J. Virol. **62:**1590–1597.
24. **Moore, R., M. Dixon, R. Smith, G. Peters, and C. Dickson.** 1987. Complete nucleotide sequence of a milk-transmitted mouse mammary tumor virus: two frameshift suppression events are required for translation of *gag* and *pol*. J. Virol. **61:**480–490.
25. **Nevo, E., and A. Beiles.** 1991. Genetic diversity and ecological heterogeneity in amphibian evolution. Copeia **3:**565–592.
26. **Papas, T. S., J. E. Dahlberg, and R. A. Sonstegard.** 1976. Type C virus in lymphosarcoma in northern pike (*Esox lucius*). Nature (London) **261:**506–508.
27. **Perbal, B. V.** 1988. A practical guide to molecular cloning, 2nd ed., p. 438–440. John Wiley & Sons, New York.
27a. **Power, M. D., P. A. Marx, M. L. Bryant, M. B. Gardner, P. J. Barr, and P. A. Luciw.** 1986. Nucleotide sequence of SRV-1, a type D simian acquired immune deficiency syndrome retrovirus. Science **231:**1567–1572.
27b. **Quérat, G., G. Audoly, P. Sonigo, and R. Vigne.** 1990. Nucleotide sequence analysis of SA—OMVV, a visna-related ovine lentivirus: phylogenetic history of lentiviruses. Virology **175:**434–447.
28. **Renne, R., E. Friedl, M. Schweizer, U. Fleps, R. Turek, and D. Neumann-Haefelin.** 1992. Genomic organization and expression of simian foamy virus type 3 (SFV-3). Virology **186:**597–608.
29. **Repaske, R., P. E. Steele, R. R. O'Neill, A. B. Rabson, and M. A. Martin.** 1985. Nucleotide sequence of a full-length human endogenous retroviral segment. J. Virol. **54:**764–772.
30. **Sagata, N., T. Yasunaga, J. Tsuzuku-Kawamura, K. Ohishi, Y. Ogawa, and Y. Ikawa.** 1985. Complete nucleotide sequence of the genome of bovine leukemia virus: its evolutionary relationship to other retroviruses. Proc. Natl. Acad. Sci. USA **82:**677–681.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., vol. II, p. 9.31–9.55. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Schwarz, D. E., R. Tizard, and W. Gilbert.** 1983. Nucleotide sequence of Rous sarcoma virus. Cell **32:**853–869.
33. **Smyth, D. R., P. Kalitsis, J. L. Joseph, and J. W. Sentry.** 1989. Plant retrotransposon from *Lilium henryi* is related to Ty3 of yeast and the *gypsy* group of Drosophila. Proc. Natl. Acad. Sci. USA **86:**5015–5019.
34. **Sonigo, P., C. S. Barker, E. Hunter, and S. Wain-Hobson.** 1986. Nucleotide sequence of Mason-Pfizer monkey virus: an immunosuppressive D-type retrovirus. Cell **45:**375–385.
35. **Stephens, R., J. Casey, and N. Rice.** 1986. Equine infectious anemia virus gag and pol genes: relatedness to visna and AIDS virus. Science **231:**589–594.
36. **Swofford, D. L.** 1991. PAUP: phylogenetic analysis using parsimony, version 3.1. Illinois Natural History Survey, Champaign. (Computer program.)
37. **Tristem, M., T. Myles, and F. Hill.** 1995. A highly divergent retroviral sequence in the tuatara (*Sphenodon*). Virology **210:**206–211.
38. **Xiong, Y., and T. H. Eickbush.** 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. **9:**3353–3362.
39. **Yamamoto, T., R. K. Kelly, and O. Nielson.** 1983. Epidermal hyperplasias of northern pike (*Esox lucius*) associated with herpesvirus and C-type particles. Arch. Virol. **79:**255–272.